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Research Article

THE EVALUATION OF SINGLET OXYGEN QUENCHING AND SUNSCREEN ACTIVITY OF CORN COB EXTRACT

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ABSTRACT

The corn cob waste has been reported to have antioxidant activity. Active compound such as antioxidant has been considered as potential sunscreen resources. The objectives of this research were to determine singlet oxygen quenching and sunscreen activity of corn cob. Corn cob was extracted using ethanol 20, 40, 60 and 80% by reflux for 2h at 78°C. The singlet oxygen quenching activity was evaluated by photooxidation of linoleic acid. Singlet oxygen quenching activity was conducted using linoleic acid as substrate containing erythrosine as a sensitizer and exposed under continuous illumination (4000 lux) with the fluorescent light for up to 5h. The sunscreen activity was evaluated by sun protection factor (SPF) using spectrophotometry UV-Vis. Ethanol extract 80% (E80) shows the highest total phenolic content followed with E60, E40 and E20. The result shows that the lowest singlet oxygen quenching activity was E20 (15.63%), and the highest was E80 by 65.63% percentage of inhibition. SPF value of E20, E40, E60, and E80 at concentration 200µg/mL were 7.52; 12.24; 15.81 and 17.78, respectively. SPF value increase with the increasing of concentration, total phenolic content and singlet oxygen quenching activity. The conclusion of this research was corn cob extract possess phytochemical compound having potency as singlet oxygen quencher and sunscreen active compound.

Keywords: corn cob, phenolic phytochemical, singlet oxygen quenching, sunscreens

INTRODUCTION

Sun light is source of energy. It is very important to organism in the world and human being. Although sun light possesses good benefit, over exposed, however, cause skin damage, such as sun burn, skin cancer and stress oxidative. Approximatly 50% of sun light which reach into the earth surface are visible light (400-800nm), 40% infrared radiation, IR (1300-1700nm), and 10% of ultraviolet radiation, UV (10-400nm).

Sun light exposure on skin can cause photochemical reaction such as photooxidation which produce Reactive Oxygen Species (ROS) such as superoxide anion, singlet oxygen molecule and hydroxyl radicals. Singlet oxygen is a ROS which electrophilic non radicals (Min and Boff, 2002; Choe *et al.,* 2005). However, singlet oxygen influences the oxydation reaction specifilcaly by attaching directly to high electron molecule without the presence of free radicals. Oxydation of biologycal compound induced by singlet oxygen related to many pathologycal activity such as pigmentation, chatarac, ageing and cancer (Davies dan Goldberg, 1987; Shahidi, 1997; Haliwell dan Guttridge, 2001). Cockell and Knowland (1999) reported that photooxidation cause DNA, cellular molecule, essential protein, amino acids and lipid membrane damage thus increase free radicals formation in skin.

Sunscreens are chemicals that provide protection against adverse effect of solar and particular UV radiation. Natural substances extracted from plants have been recently considered as potential sunscreens resources because of their ultraviolet radiation absorption in the UV region and their antioxidant activity.

The last ten years, there were increasing of preferences to use antioxidant in sunscreen to add photoprotective benefit. The antioxidant from natural sources exhibit new possibility to inhibit diseases mediated by UV light (Bonina *et al.,* 1996; Saija *et al.,* 1998). Corn (*Zea mays* L.) is one well known of crops and cultivated in developing country. The utilization of corn seeds as food material resulting corn cob as waste. Corn cob is a phenolic phytochemical containing biomass which recommended to be used as active antioxidant compound (Lumempouw *et al*., 2012). Hossain *et al.* (2006) identified flavonoids from flavonol group such as quercetine and its glycoside from corn. Despite antioxidant activity of corn cob had been published, there are no data available concerning the singlet oxygen *quenching* and sunscreen activity from corn cob extract. The objectives of this study were to examine the singlet oxygen *quenching* and sunscreen activity of ethanolic corn cob.

MATERIAL AND METHOD

Sample used in this research was dried corn cob hybride variety which purchased from local farm in Gorontalo. Chemicals used in this research were ethanol, acetic acid, chloroform, potassium iodide, sodium carbonate, Folin-Ciocalteure agent purchased from Merck (Darmstat, Germany) Gallic acid was purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin).

Procedure

Extraction of corn cob

Corn cob was extracted using ethanol 20, 40, 60 and 80%. Extraction was done by reflux. Twenty five grams corn cob were added to 500mL round flask, then added with 250mL ethanol up to the sample immersed, then heated for 2h at 78°C. Filtrate were filtered and evaporated using evaporator to yield crude extract.

Determination of total phenolic content

Total phenolic content in corn cob extract were examined using Folin-Ciocalteu method (Jeong *et al*., 2004). The absorbances were read at λ 750nm using spectrophotometer. Total phenolic content were expressed as mg equivalent of gallic acid mg/kg extract.

Determination of singlet oxygen quenching activity on photooxydation of linoleic acid

Determination of singlet oxygen quenching activity from corn cob extract on linoleic acid were evaluated using Lee *et al.* (1997) method, with modification. The effect of extract on singlet oxidation of linoleic acid 0.03M using concentration 1000μg/mL which prepared in ethanol containing 5μg/mL of erythrosine as senzitiser. Ten mililitre of samples were taken from the mixture, put in serum with capasity of 30mL equipped with rubber stopper and aluminium foil. The bottle were placed and stored in light box (70x50x6 cm) with fluorescent light intensity were 4000 lux for 5h. Peroxide value were examine using AOCS (1990) method. The same procedure were done for condition without light.

Identification of sunscreen active component

Identification of sunscreen active component was evaluated using UV and IR spectrophotometer method. The absorbance of corn cob extract 100μg/mL was read at λ 200 to 400nm using Spectrophotometer UV-Vis PG instrument T80. Furthermore, extract was prepared for analysis of IR profile using Shimadzu FTIR 8201 PC.

Determination of SPF value *in vitro*

Determination of sunscreen activity were conducted by examining SPF *in vitro* value using spectrophotometer (Spectrophotometer UV-Vis PG instrument T80) (Mansur *et al*., 1986; Walters *et al.,* 1997). Corn cob extract was made 50-200μg/mL in water and ethanol mixture. Absorbance curve of extract solution were made in 1cm cuvette, wavelenght of 290 to 320nm with 5nm interval. Absorbance of the solution shows the effect of substance which absorb or reflect UV light in solution. Mansur *et al.* (1986) develop simple mathematical equation to calculate SPF value.

$$SPF = CF \times \sum_{290}^{320} EE\,(\lambda) \times I\,(\lambda) \times absorbance$$

Note: CF: Correction factor (10), EE: erythermal eficiency, λ : wave length, I: sun light spectrum simulation and Abs : sunscreen product absorbance.

Table I. Normalization product function which used to calculate SPF (Sayre *et al.*, 1979)

Wavelength (λ, nm)	EE x I (Normalization)
290	0.015
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0837
320	0.018
Total	1

Table II. Characreristic peak band on IR spectra for different group

Group	Wave number region (cm^{-1})	Characteristic
O-H	3600-3200	H- bonded broad and strong
-CH-	2924	Methin (-CH-)
	2855	
	1373	Methylene group ($-CH_2-$) and methyl (CH_3) strenghtened with band
C=O	1697	Strong and sharp absorbance at 1697 cm^{-1} shows the presence of carbonyl group
-C-C-	1604 and 1512	Aromatic ring
C-O	1300-1100	Alcohol and ether strong, ester two bands or more

EE x I value is constant. The values were determined by Sayre *et al.* (1979) as shown in table I. Examination of SPF using spectrophotometer was carried out by making absorbance curve of solution in 1cm cuvette, at the wavelength of 290 to 320nm. Absorbance represents the effect of sun screen active component which absorb or refrect UV light in solution. Furthermore, absorbances were read at 5nm interval from 290 to 320nm.

The same procedure were done to extract in ethanol 20%, 40%, 60% and 80%. Positive control is sun screen with SPF 15.

RESULTS AND DISSCUSSION

Extraction and total phenolic content

Extraction using ethanol 60% produce the highest yield of extract (2.49%), followed with ethanol 40% (2.18%), ethanol 60% (1.55%) and ethanol 80% (1.77%). Obtained extracts were determined its total phenolic content using Folin-Ciocalteu method. The principle of this method based on reduction ability on phosphomolibdate-phosphotungstat from Folin-Ciocalteu which form blue color, thus it can be determine by spectrophotometer. Determination of total phenolic content were expressed as gallic acid μg/mL extract.

The results showed that total phenolic of corn cob extract in the range 38.98 to 73.06μg/mL, with average 58.73μg/mL (Figure 1). Corn cob extract from ethanol 20% (E20) and 40% (E40) possess the lowest total phenolic content followed by that of ethanol 60% (E60), and the highest total phenolic content were corn cob extract from ethanol 80% (E80). Low concentration of ethanol resulting low total phenolic content in corn cob (Figure 4). The intensity of color indicated total phenolic content in extract. It means that corn cob extract with ethanol 80% solvent possess high intensity of blue color compared to that of

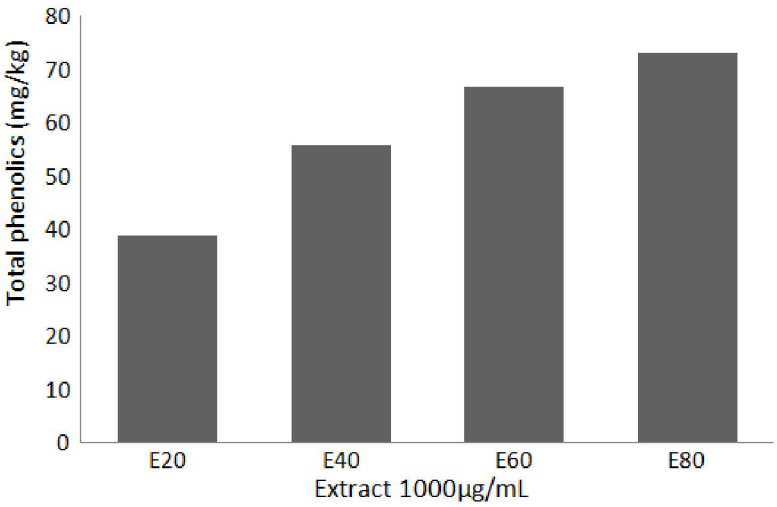

Figure 1. Total phenolic content of corn cob extract (E20:ethanol 20%, E40: ethanol 40%, E60: ethanol 60% andE80: ethanol 80%

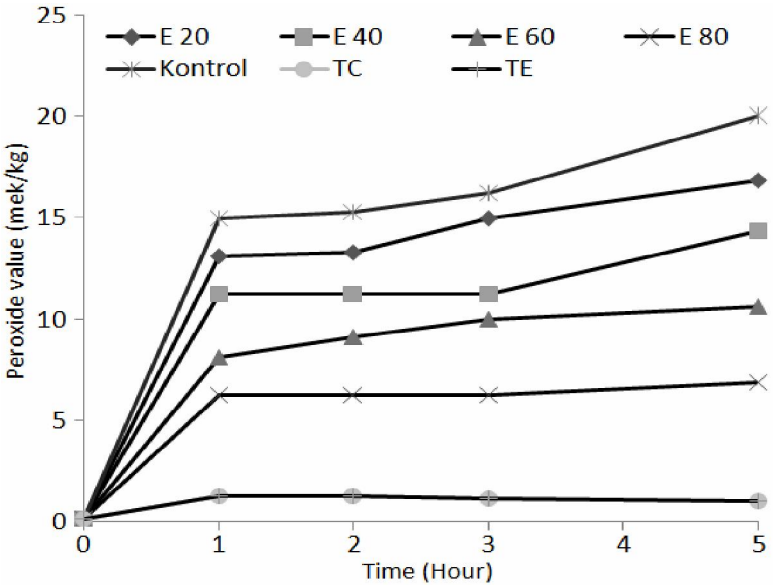

Figure 2. Singlet oxygen quenching activity of corn cob extract on linoleic acid photooxydation

E60, E40, and E20% as solvent. High total phenolics content in extract resulting high antioxidant activity. Phytochemicals such as phenolic, flavonoids and tannins are usually having antioxidant acivity. Fidrianny *et al.* (2012) reported that ethanol extract of *Psidium guajava* possessed high total phenolic content showed high antioxidant activity compared with other extracts which have less total phenolic content.

Singlet oxygen quenching of corn cob

The effects of 1000µg/mL E20, E40, E60 and E80 extracts on peroxide value of linoleic acid which exposed with 4000lux light are presented in figure 2. E80 extract showed the highest effect in singlet oxygen quenching followed by E60, E40 and E20 for 5h fluorescent light exposured ($p<0.05$). Erythrosine which was exposed with natural light (negative control)

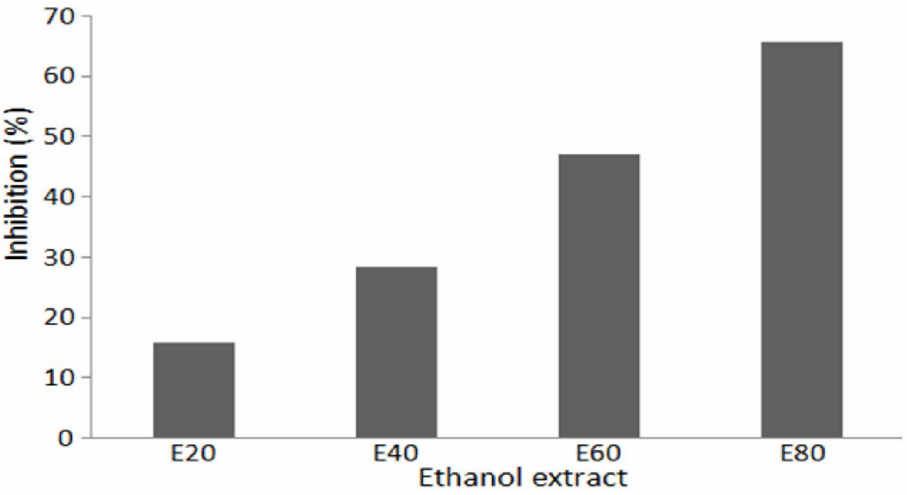

Figure 3. Singlet oxygen inhibition from various corn cob extract

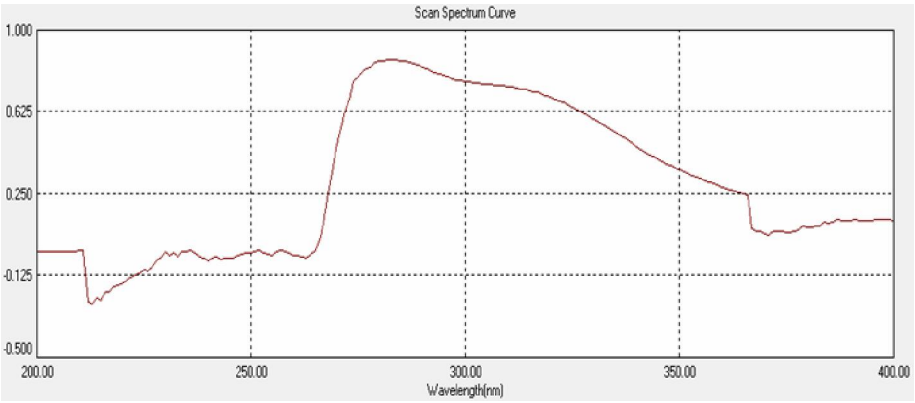

Figure 4. UV spectral of phenolic extract of corn cob

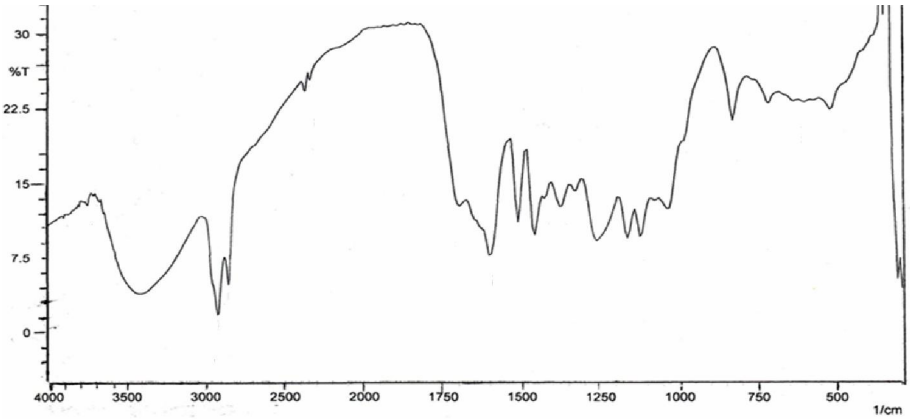

Figure 5. Infrared spectral of phenolic extract of corn cob

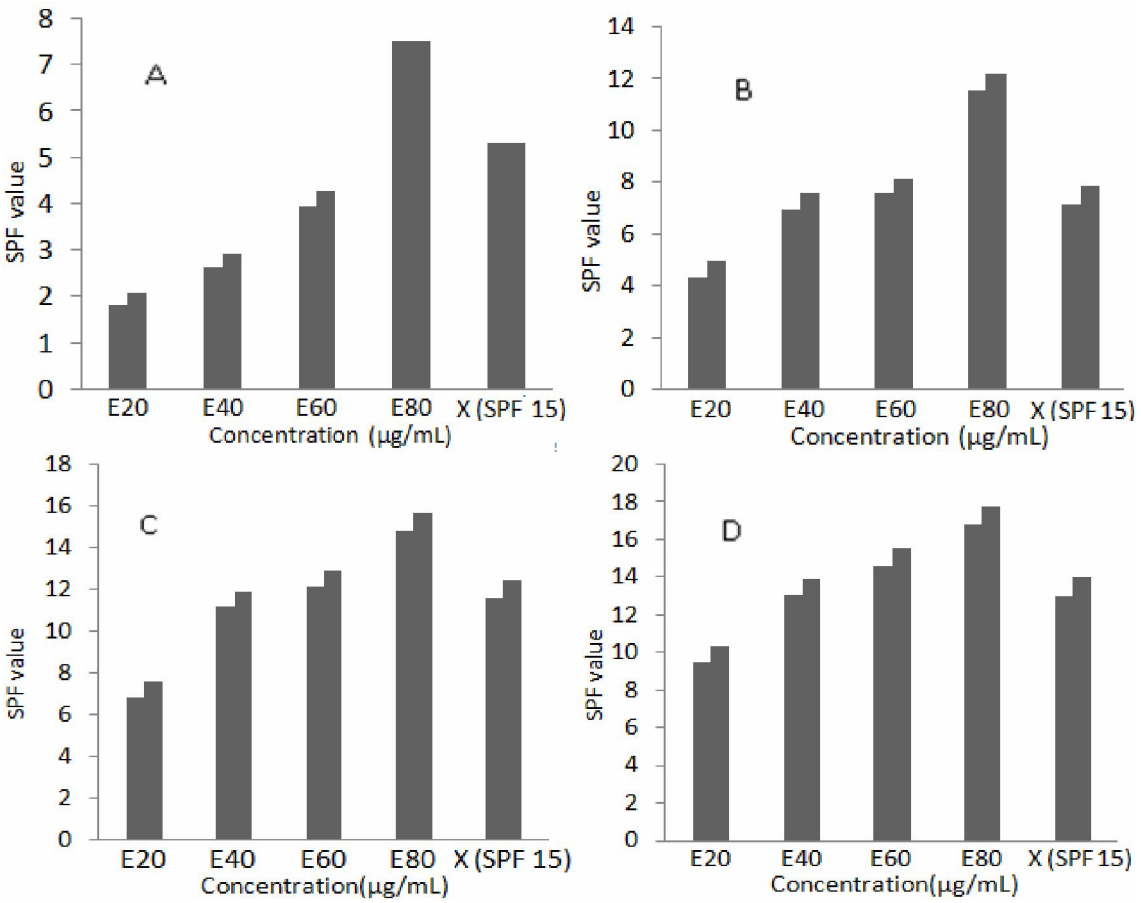

Figure 6. SPF value of various corn cob extract concentration (A: 50µg/mL, B: 100µg/mL), C: 150µg/mL, D: 200µg/mL and $X_{SPF\ 15}$ (positive control).

shows peroxide value changes which increase during 5h light exposure. It means that erythrosine act as photooxidation initiator of linoleic acid, its proved with the increases of peroxide value of linoleic acid during 5h light exposure. In contrast linoleic acid exposured with light without sensitizer (TE) and without light (TC) did not show signifficant peroxide value changes. It means that linoleic acid whithout erytrhosine or riboflavine did not produce singlet oxygen from triplet oxygen. Min and Boff (2002) stated that singlet oxygen can be produced by triplet oxygen with the presence of light and sensitizer. The presence of sensitizer, increase oxidation reaction, make it absorb energy from light to form hydroperoxide by photooxidation reaction. Photooxidation of singlet oxygen in linoleic acid generate hydroperoxide at conjugated double bond 9-OOH and 13-OOH and unconjugated 10-OOH and 12-OOH. Autooxidation of triplet oxygen on linoleic acid produce hydroperoxide at position conjugated 9-OOH and 13-OOH (Frankel, 1987).

Linoleic acid with erythrosine (control) showed the highest peroxide value compared to that of extract treatment. Data proved that corn cob extract decrease peroxide value, or possess ability to stabilize singlet oxygen. The effect of 1000µg/mL E80 showed more active as singlet oxygen quenching followed with E60, E40 and E20. The longer exposure time, the more of singlet oxygen formed in control group, in contrast to that of corn cob extract (Figure 2).

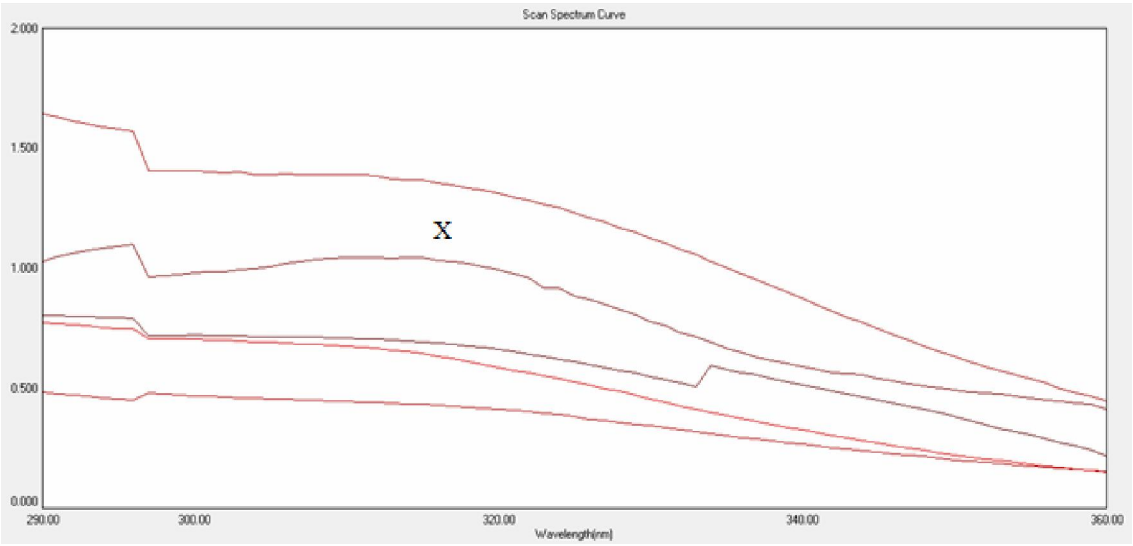

Figure 7. Absorbance spectral of 100µg/mL E20 (ethanol 20%), E40 (ethanol 40%), E60 (ethanol 60%), E80 (ethanol 80%) and X (positive control, X SPF 15)

The obtained peroxide values were used to calculate the precentage of singlet oxygen inhibition (Figure 3). Corn cob extract using ethanol 80% as solvent showed the highest singlet oxygen inhibition followed with E60, E40 and E20. Singlet oxygen inhibition of E80, E60, E40 and E20 were 15.63; 28.13; 46.88 and 65.63% respectively. E80 was effective extract to stabilize singlet oxygen generation from erythrosine sensitizer as showed by the increasing of singlet oxygen inhibition. High concentration of phenolic content and free radicals scavenging activity correlated with high singlet oxygen quenching activity of corn cob.

The formation of singlet oxygen mainly generated with the presence of photochemical sensitizer. Chlorophyl, riboflavin, myogoblin, porfirins, food and textile colorant act as photosensitizer and may absorb light energy and transferred to triplet oxygen to form singlet oxygen (Foote *et al*., 1970). Photooxydation research on lipids is an example to explain mechanism of singlet oxygen formation on human skin. Human skin consist of natural sensitizer namely melanine. Melanine is natural skin protector, however, when it exposed with light (uv) for considerable time with high intensity, it may act as sensitizer, thus generate oxidation reaction which produce reactive oxygen species.

Identification of sunscreen active component

Sayre *et al*. (1990) stated that the ability radiation of active component in extract to absorb UV associates with sun screen activity. Light absorbtion can be evaluated by measuring absorbancy at wavelenght 290-320nm. The increasing of absorbance at the wavelenght 320nm correlate to the activity to absorb UV radiation. Effectivity of sun screen was evaluated by spectrophotometry (Figure 4). Analysis using UV spectophotometer at wavelenght 200-400nm indicating that the max absorbtion were at 280 and 312nm. Corn cob extract absorb of UVA and UVB act (290-360nm). This result concluded that phenolic extract from corn cob contains as active component of sun screen to UVA and UVB, and act as singlet oxygen *quenching*.

Maximum peak of corn cob extract at 280nm was due to the presence of phenolic groups. Maximum absorbtion at 312nm could be due to the presence of flavonoids. Mabry *et al*. (1970) reported that flavon and flavonols showed two peaks at 240 and 400nm, one peak at range 240-280nm, and the other one at range of 300-380nm. IR analysis (Figure 5) support the presence of phenolic groups in extract. Analysis of IR spectral shows that there are wide and strong band at 3310cm^{-1} means the

presence of OH group, followed with strong bands at 2924cm^{-1} and 2855cm^{-1} shows the presence of methine (-CH-), methylene group (-CH_2-) and methyl (CH_3) strenghtened with band at 1373cm^{-1}.

Strong and sharp absorbance at 1697cm^{-1} shows the presence of carbonyl group (C=O). Absorbance band at 1604 cm^{-1} and 1512cm^{-1} indicate the presence of aromatic ring (–C-C-) (Silverstein *et al*., 1991) and followed with band at 1300-1100cm^{-1} that indicate C-O group of ether, in which band in this frequency was specific for aryl ether. Based on IR spectral analysis, it can be concluded that phenolic extract from corn cob possess hydroxy (OH), Carbonyl (C=O), aromatic (C=C), ether (R-O-R), methyle (-CH-) metylene (-CH_2-) and methyl (CH_3) groups. This data proved that corn cob extract consist of phenolics such as simple phenol, flavonoids and tannin swhich possess aromatic ring and shows synergism effect as active component of sun screen.

Phenolic groups can inhibit induction of UV generation, free radicals and lipid peroxidation which involved in pathologic condition such as photoageing and skin cancer. Saija *et al*. (2000) reported that simple phenol such as caffeic acid and ferulic acid effectively protect human skin from UVB radiation that cause erytherma. Ferulic acid strongly proved can absorb UV radiation and used as sun screen (*photoprotective*) in some lotion and sun screen cream. Another research, Inal *et al*. (2001) reported that quercetin can protect skin with its antioxidant system, that is peroxidation glutation, reductase glutation, catalase and superoxide dismutase activity on UVA radiation on rats skin. Consumption of quencetine inhibit UVB radiation damage by imunosupresition in SKH-1 rats (Steerenberg *et al.,* 1997). Furthermore, tannins is poly-phenolics having antioxidant potency protect damage skin caused by free radicals which generated with UV light and decrease skin cancer risk and ageing (Ho, 2001)

Determination of SPF *in vitro*

Determination of sun screen effectivity was done by determine SPF value *in vitro* using UV spectrophotometer at wavelength of 290 to 320nm. SPF were determining quantitatively from active component as sun screen that prevent sunburn and another skin damage. SPF was evaluated with Mansur equation. The results showed that SPF value of different four concentrations of corn cob extract were between 1.99 and 17.78 (Figure 6). The results showed that extract E80 possess the highest SPF followed by E60, E40 and E20 at any level of concentrations.

High concentration of extract possessed high SPF value (Figure 6). It means that corn cob extract possess ability to protect skin from sun light especially UV-B. SPF value of E80, E60 and E40 at concentration 200μg/mL categorized as moderate group. According *Food and Drug Administration* (1999), sun screen potency measurement based on sun protector factor (SPF) is as follow: minimum, (SPF value 2-12) moderate, (SPF between 12-30) and high, (SPF ≥ 30). SPF value of marketed sun screen were 2-70. Active sun screen component in corn cob might be phenolic and flavonoid. This compound proved to protect skin from UV radiation damage (Bonina, 1996).

Ultraviolet spectral absorbance of four phenolic extracts from corn cob and positive control (SPF 15) were figure 7. High concentration of extract showed high ability to absorb UV radiation. The result shows that sun screen activity was concentration dependent. E80 and E60 possess sun screen activity greater than marketed sunscreen X (SPF 15) as positive control at 100μg/mL, but E40 ($p>0,05$). SPF value of E80, E60, E40 and X were 12.24; 817; 7.33 and 7.91 respectively.

CONCLUSIONS

Extract E80 showed greater total phenolic content and singlet oxygen quenching activity compared to E60, E40 dan E20. The greater the concentration of corn cob extract, the greater its antioxidant activity. Extract E80 had sun screen activity and lightst SPF value.

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Edi Suryanto Email : edisuryanto@yahoo.com ABSTRACT The corn cob waste

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Active compound such as antioxidant has been considered as

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The objectives of this research were to determine singlet

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Corn cob was extracted using ethanol 20, 40, 60

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The singlet oxygen quenching activity was evaluated by photooxidation

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Ethanol extract 80% (E80) shows the highest total phenolic

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activity was E20 (15.63%), and the highest was E80 by

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SPF value of E20, E40, E60, and E80 at

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SPF value increase with the increasing of concentration, total

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The conclusion of this research was corn cob extract

Research Article Volume 24 Issue 4 (2013) 267 Indonesian J. Pharm. Vol. 24 No. 4 : 267 – 276 ISSN-p : 2338-9427 DOI: 10.14499/indonesianjpharm24iss4pp267 THE EVALUATION OF SINGLET OXYGEN QUENCHING AND SUNSCREEN ACTIVITY OF CORN COB EXTRACT Edi Suryanto 1 *, Lidya Irma Momuat 1 , Adithya Yudistira 2 , Frenly Wehantouw 2 1 Department of Chemistry, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado, Indonesia. 95115 2 Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado, Indonesia. 95115 Submitted: 06-07-2013 Revised: 20-08-2013 Accepted: 22-09-2013 *Corresponding author Edi Suryanto Email : edisuryanto@yahoo.com ABSTRACT The corn cob waste has been reported to have antioxidant activity. Active compound such as antioxidant has been considered as potential sunscreen resources. The objectives of this research were to determine singlet oxygen quenching and sunscreen activity of corn cob. Corn cob was extracted using ethanol 20, 40, 60 and 80% by reflux for 2h at 78 o C. The singlet oxygen quenching activity was evaluated by photooxidation of linoleic acid. Singlet oxygen quenching activity was conducted using linoleic acid as substrate containing erythrosine as a sensitizer and exposed under continuous illumination (4000 lux) with the fluorescent light for up to 5h. The sunscreen activity was evaluated by sun protection factor (SPF) using spectrophotometry UV-Vis. Ethanol extract 80% (E80) shows the highest total phenolic content followed with E60, E40 and E20. The result shows that the lowest singlet oxygen quenching activity was E20 (15.63%), and the highest was E80 by 65.63% percentage of inhibition. SPF value of E20, E40, E60, and E80 at concentration 200µg/mL were 7.52; 12.24; 15.81 and 17.78, respectively. SPF value increase with the increasing of concentration, total phenolic content and singlet oxygen quenching activity. The conclusion of this research was corn cob extract possess phytoch

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